Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

By way of the above amendments, applicants have amended claims 1, 2, 5, 7, 8, 9, 17, 19, 20, 23, and 26; cancelled claims 4 and 29-58 without prejudice; and introduced new claims 59-74. New claim 59 is essentially claim 4 written in independent form (including recitation of an inducible promoter). New claims 60-73 correspond to claims 2, 3, 11, 12, 16-23, 27, and 28, respectively, but depend from claim 59 rather than claim 1. New claim 74 corresponds to original claim 1 but is limited to the identification of a gene that alters the lifespan of an organism. No new matter has been introduced by these amendments.

Claims 1-3, 5-28, and 59-74 remain pending, with claims 13-15, 24, and 25 being withdrawn. No additional claims fees are required, because only three independent claims are presented and only 43 total claims are presented (which is less than the 58 total claims previously paid for).

The rejection of claims 1, 4, 7, 19, 23, and 26 under 35 U.S.C. § 112 (second paragraph) for indefiniteness is respectfully traversed in view of the above amendments.

Claims 1, 19, and 23 now recite the term "replicative lifespan". Replicative lifespan, as defined within the specification on page 3, line 31-33 through page 4, lines 1-2, refers to the number of times a mother (yeast) cell divides before dying. This term, therefore, is not indefinite.

The rejection of claim 4 is moot and does not apply to new claim 59, which recites use of an "inducible promoter that is responsive to growth medium conditions." Support for this amendment is found page 9, lines 4-8 of the specification.

Claim 7 now recites the term "HO-endonuclease promoter." Support for this limitation is found on page 9, line 34-35 through page 10, lines 1-2. Additionally, the nucleotide sequence for the *S. cerevisiae* HO-endonuclease promoter is provided on page 10 lines 8-31.

Claim 26 has been amended to depend from claim 59 and to instead recite with proper antecedent basis to the "two chimeric genes" of claim 59.

For all these reasons, the rejection of claims 1, 4, 7, 19, 23, and 26 for indefiniteness should be withdrawn.

The rejection of claims 1-12, 16-23, and 26-28 under 35 U.S.C. §112 (1st paragraph) for lack of enablement is respectfully traversed in view of the remarks below.

The U.S. Patent and Trademark Office ("PTO") has asserted that because the assay of the present invention has only been described for practice in yeast, it cannot be used to extrapolate results for any other organism. Applicants respectfully disagree for the reasons set forth in the accompanying Declaration of David S. Goldfarb under 37 C.F.R. § 1.132 ("Goldfarb Declaration") at ¶¶6-8.

There exists ample scientific evidence that the study of yeast replicative lifespan is relevant to the lifespan of other animals, including vertebrates and mammals. This is particularly true for mechanisms of aging that are evolutionarily conserved among disparate organisms, which are known as "public" mechanisms of aging. Goldfarb Declaration at ¶6.

The best understood public aging phenomenon is caloric restriction (CR). Goldfarb Declaration at ¶7. CR, which generally involves moderate reductions in food intake, has been shown to extend the lifespan of yeasts, fruit flies, worms, and mice. *Id.* CR is an "ancient" phenomenon that evolved in single-cell eukaryotes millions of years before the appearance of metazoan animals. *Id.* Landmark studies on the molecular mechanism of CR in yeast have led to breakthroughs in the understanding of CR in metazoan animals. *Id.* The existence of genes that regulate lifespan via CR was first demonstrated in yeast, with the discovery that overexpression of the sirtuin protein, Sir2p, extends yeast replicative lifespan . *Id.* Genetic studies have since extended these results to show that sirtuins regulate lifespan in the worm, *C. elegans,* and in the fruit fly, *D. melanogaster*. *Id.* Because the role of the sirtuins in the lifespan of evolutionarily distinct animals was first discovered in yeast, it is reasonable to utilize yeast to measure the effects of genetic variation and/or environmental conditions for these "public" mechanisms of aging.

The pursuit and discovery of small molecules that can delay age-related diseases and increase lifespan in mammals was directed by early studies on the role of yeast Sir2p in replicative lifespan. Goldfarb Declaration at ¶8. The study of mammalian Sir2p-like proteins has focused attention on the sirtuins as potential pharmacological targets to treat the major diseases of aging. *Id.* An *in vitro* biochemical screen for small molecules that activate the mammalian Sir2p homolog, SIRT1, led to the finding that the plant compound resveratrol may act as a "caloric restriction mimetic." *Id.* Resveratrol increases the lifespan of yeast, fruit flies, and a vertebrate fish. *Id.* Resveratrol also mitigates several deleterious physiological effects of a high-calorie diet in mice. *Id.* A separate study in mice has also demonstrated that resveratrol protects animals against diet-induced obesity and insulin

resistance. *Id.* Other examples of public mechanisms of aging are reviewed by Dilova (*see* Exhibit 2 to the Goldfarb Declaration, at 754-756), and include the TOR signaling pathway and glucose-sensing pathways. *Id.*

The results described above prove that the study of yeast replicative lifespan is directly relevant to public aging and senescence mechanisms that are conserved in higher animals. Goldfarb Declaration at ¶8. As concluded by Dilova: "Owing to the remarkable evolutionary conservation in longevity regulating proteins and signaling pathways, studies of longevity regulation in model organisms have contributed and will continue to provide a wealth of information relevant to *human* aging and age-associated diseases." *Id.* (citing Exhibit 2 at 762 (emphasis added). Therefore, the study of yeast replicative lifespan can provide meaningful identification of "public" mechanisms of aging with respect to both environmental and genetic modifications. *Id.*

The PTO also asserts that the examples provided do not teach how such an assay can identify environmental stimuli, genes, and/or combinations of the two that alter lifespan of any organism because the examples are limited to teaching that SIR2 and SGS1 genetic modifications, which were already known in the art to alter yeast replicative lifespan. Again, applicant respectfully disagrees.

For the reasons described in the present application and as evidenced by the accompanying Goldfarb Declaration at ¶¶10-15, the present invention describes a fast, high throughput, and less labor-intensive replicative lifespan assay that will facilitate the continued use of yeast as a model organism for the study of aging. Goldfarb Declaration at ¶11.

Additional validation studies have been performed that demonstrate the assay is indeed capable of identifying genetic modifications, environmental stimuli, and the combination thereof that may alter replicative lifespan. Goldfarb Declaration at ¶¶11-14.

As one example, yeast replicative lifespan in the assay of the present invention is sensitive to the presence or absence of *SGS1* expression, where introduction of a plasmid-borne copy of wild-type *SGS1* into a deletion strain rescues the short lifespan and an otherwise identical plasmid lacking the *SGS1* gene did not rescue the lifespan defect. Goldfarb Declaration at ¶12. These results demonstrate that the assay is an efficient method for screening among systematically inserted point mutations (genetic changes that alter a single nucleotide or amino acid position) of the *SGS1* gene for those that can or cannot rescue the lifespan of an *sgs1* mutant strain. *Id.* The generation of large numbers of point mutations in genes is a standard and trivial practice. *Id.* What is not trivial is the large-scale measurement of replicative lifespan. *Id.* The method of the present invention can be applied

for any gene whose deletion causes a reduction or extension of lifespan, such as *SIR2*. *Id.* This type of analysis requires as many as one hundred or more lifespan assays, which is highly impractical when using the standard microdissection lifespan assay (*see* Goldfarb Declaration at ¶10) but relatively easily performed using the assay of the present invention. *Id.*

As another example, yeast replicative lifespan in the assay of the present invention is sensitive to the level of *SIR2* expression. While Example 1 of the present application demonstrates that deletion of the *SIR2* gene shortens yeast lifespan as measured using the DeaD assay, the accompanying Goldfarb Declaration demonstrates that yeast replicative lifespan varies in proportion to the level of *SIR2* expression. Goldfarb Declaration at ¶13. The results present in the Goldfarb Declaration show that the DeaD assay reproduces the role of *SIR2* under- and over-expression on lifespan, and that DeaD assay is capable of identifying genetic alterations that cause lifespan reduction and/or extension. *Id.*

Finally, the accompanying Goldfarb Declaration demonstrates that the yeast replicative lifespan in the assay of the present invention allows the analysis of environmental factors that influence aging. Goldfarb Declaration at ¶14. This is relevant to both "public" mechanisms as well as "private" yeast mechanisms of aging. *Id.* The results presented in the accompanying Goldfarb Declaration confirm that nicotinamide, a vitamin B_3 precursor known to inhibit Sir2p and shorten replicative lifespan, reduces yeast replicative lifespan in a dose-dependent fashion without affecting cell growth under permissive conditions (raffinose/galactose) as measured using the yeast replicative lifespan in the assay of the present invention. *Id.* It is also shown that the lifespan-shortening affect of nicotinamide was dependent on the *SIR2* gene. *Id.*

Collectively, the validation experiments described in paragraphs 12-14 of the accompanying Goldfarb Declaration demonstrate that the assay of the present invention faithfully reproduces results obtained using the standard yeast replicative lifespan assay. More importantly, these results demonstrate that the assay of the present invention is sensitive to both genetic modifications, environmental stimuli, and the combination thereof. Therefore, the methods described in the present application are enabling for the study of yeast replicative lifespan and the identification of genetic modifications, environmental stimuli, and the combination thereof that may be relevant to aging in yeast and in higher organisms that share genetically conserved aging pathways.

For the foregoing reasons, the rejection of claims 1-12, 16-23, and 26-28 for lack of enablement is improper and should be withdrawn.

The rejection of claims 1-3, 11, 12, 16, and 19 under 35 U.S.C. §102(b) as being anticipated by U.S. Patent No. 6,228,583 to Guarente et al. ("Guarente") is respectfully traversed.

Guarente teaches a method of identifying a compound that inhibits the replication and accumulation of ribosomal DNA circles. Because accumulation of these DNA circles is believed to reduce lifespan, according to Guarente inhibition of their accumulation may enhance the lifespan of a cell. Guarente utilizes transformed yeast cells that contain a plasmid containing a marker gene and an autonomously replicating sequence. There are two classes of transformed cells: stably transfected cells, where both mother and daughter cells are capable of replicating; and non-stably transected cells, where the mother cells are capable of replicating but the daughter cells cannot. Both classes of transformed cells *are required* in the Guarente method to identify a candidate compound. The Guarente method involves culturing control and test cultures under conditions whereby mother yeast cells can replicate and daughter cells cannot *and* under conditions whereby mother yeast cells and daughter yeast cells both replicate. Identification of a test compound as one that can alter lifespan requires a comparison of cells grown under *both* conditions. In contrast, claim 1 of the present application involves a comparison of control and test cultures that are grown under the same conditions, but by virtue of the transition language "consisting essentially of" the claim scope excludes performing the comparison under *two* sets of conditions as required by Guarente.

For these reasons, the rejection of claim 1 as well as claims 2, 3, 11, 12, 16, and 19 dependent thereon as anticipated by Guarente is improper and should be withdrawn.

The rejection of claims 1-3, 11-12 and 16-21 under 35 U.S.C. §103(a) as being unpatentable over Guarente in view of U.S. Patent No. 6,531,289 to Bradley et al ("Bradley") is respectfully traversed.

The teachings and deficiencies of Guarente are identified above. While Bradley teaches a method of screening for yeast cell growth where the yeast is grown in liquid media and the growth is measured by optical density of the cells in the media, as well as measuring yeast cell growth on solid plates by colony formation, Bradley fails to overcome the above-noted deficiencies of Guarente. Because the PTO has not identified how Bradley overcomes these deficiencies, the rejection of claims 1-3, 11-12 and 16-21 for obviousness over Guarente in view of Bradley is improper and should be withdrawn.

Applicants further submit that new claim 59 and claims 5-9 and 60-73 dependent thereon are allowable over Guarente, alone or in combination with Bradley, because original claim 4 was not rejected over Guarente or the combination of Guarente and Bradley. As noted above, the limitations of original claim 4 have been introduced into new claim 59.

Applicants further submit that new claim 74 is allowable over Guarente, alone or in combination with Bradley, because neither of these references teach or suggest using the Guarente system to assess a gene that alters the lifespan of an organism.

In view of all of the foregoing, applicant submits that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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